*Original Article*
http://mjiri.iums.ac.ir
**Medical Journal of the Islamic Republic of Iran (MJIRI)**

*Med J Islam Repub Iran.* 2024 (18 Nov);38.133. https://doi.org/10.47176/mjiri.38.133

# The Effect of Some Micronutrients Multi-Supplementation on Thyroid Function, Metabolic Features and Quality of Life in Patients Treating With Levothyroxine and Vitamin D: A Double-blind, Randomized Controlled Trial

Mehrnaz Nikkhah[1], Mohammadreza Vafa[1]*, Behnaz Abiri[2], Fatemeh Golgiri[3], Parvin Sarbakhsh[4], Fariba Alaei-Shahmiri[3]

Received: 22 Jun 2024 Published: 18 Nov 2024

## Abstract

**Background:** It has been reported that reducing inflammatory damage and providing essential nutrients can improve thyroid function. Although sufficient clinical evidence does not support the routine prescription of nutritional supplements except for vitamin D therapy, as part of hypothyroidism treatment. We aimed to investigate the effects of supplementation with certain micronutrients known as essential for normal thyroid function on thyroid function, metabolic manifestations, and quality of life in patients with hypothyroidism.

**Methods:** In the current randomized controlled trial, we randomly assigned 104 patients with hypothyroidism receiving levothyroxine and vitamin D to either supplementation with 200 µg selenium, 15 mg zinc, 250 mg magnesium, 2500 IU vitamin A, 120 mg vitamin C, and 100 IU vitamin E per day (LT4VDS group) or placebos (LT4VDP group) for 8 weeks. Serum thyroid-stimulating hormone (TSH), free thyroxine (fT4), anti-thyroid peroxidase antibody (TPO-Ab), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), fasting blood sugar (FBS), fasting insulin, homeostatic model assessment for insulin resistance (HOMA-IR), C-reactive protein (CRP), as well as blood pressure, and anthropometric values, were measured before and after intervention. The 36-item short-form survey, the International Physical Activity Questionnaire, and 2 food recalls were completed. Thyroid function test, metabolic factors, and quality of life indices were compared between the 2 groups after the intervention, using analysis of covariance tests, with robust standard errors and intention to treat analysis, "multiple imputation method," adjusted for covariates.

**Results:** In the LT4VDP group, higher postintervention values of FBS (98.5 [85.7, 106.5] in LT4VDP group vs 89 [82.5, 95.7] in LT4VDS group; $P = 0.012$; effect size, 0.083), and HOMA-IR (2.1 [1.3, 3.8] in LT4VDP group vs 1.6 [1.1, 2.4] in LT4VDS group; $P = 0.031$; effect size, 0.053) were observed. Intention to treat analysis (n = 95 participants) showed similar results regarding FBS. In the LT4VDP group, a marginal increase regarding CRP (Δ: 1 [-1, 1] in LT4VDP group vs –0.6 [–1, 1] in LT4VDS group; $P = 0.051$), and Insulin (Δ: 2.9 [0.4, 6.1] in LT4VDP group vs 1 [–0.5, 2.3] in LT4VDS group; $P = 0.042$) were observed, whereas in the LT4VDS group the physical quality of life partially improved (Δ: 0.2 [9.1] in LT4VDP group vs 3.6 [6.3] in LT4VDS group; $P = 0.044$, effect size, 0.012). Between-group comparison of difference values did not show significant results regarding other outcomes, including TSH, fT4, TPO-Ab, mental quality of life, TG, TC, LDL, HDL, and blood pressure.

**Conclusion:** An 8-week supplementation with the nutrients above may affect insulin resistance and quality of life in patients with hypothyroidism; additional clinical trials are recommended.

**Keywords:** Levothyroxine, Quality of Life, Dietary Supplement

***Conflicts of Interest:*** *None declared*
***Funding:*** *Iran University of Medical Sciences has supported this study*



***Corresponding author:*** *Dr Mohammadreza Vafa, vafa.m@iums.ac.ir*

1. Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
2. Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran
4. Department of Statistics and Epidemiology, School of Public Health, Tabriz University of Medical Sciences, Tabriz, Iran

***↑What is "already known" in this topic:***
It has been reported that reducing inflammatory damage and providing essential nutrients improve thyroid function, although sufficient clinical evidence does not support the routine prescription of nutritional supplements as part of hypothyroidism treatment except for regular vitamin D therapy.

***→What this article adds:***
In hypothyroid patients, concurrently receiving regular doses of levothyroxine and vitamin D the supplementation of selenium, zinc, magnesium, vitamin A, vitamin C, and vitamin E at physiological doses for 8 weeks may affect certain clinical outcomes, including glycemic status.



[ DOI: 10.47176/mjiri.38.133 ]

*Cite this article as*: Nikkhah M, Vafa M, Abiri B, Golgiri F, Sarbakhsh P, Alaei-Shahmiri F. The Effect of Some Micronutrients Multi-Supplementation on Thyroid Function, Metabolic Features and Quality of Life in Patients Treating With Levothyroxine and Vitamin D: A Double-blind, Randomized Controlled Trial. *Med J Islam Repub Iran.* 2024 (18 Nov);38:133. https://doi.org/10.47176/mjiri.38.133

## Introduction

Hypothyroidism (HT) is characterized by an elevated level of serum thyroid stimulating hormone (TSH), accompanied by a decreased level of free thyroxine (fT4) outside the reference range (1). HT has been linked to impaired health-related quality of life and increased risk of metabolic abnormalities (2). Following a decrease in global iodine deficiency, autoimmune Hashimoto's thyroiditis (AIHaT), recognized by elevated levels of serum anti-thyroid peroxidase antibody (TPO-Ab) is regarded as the leading cause of primary HT (3). Even after receiving appropriate weekly doses of levothyroxine, 15% of patients continue to experience nonspecific HT manifestations, such as fatigue, weight gain, and deterioration in health (4). Previous research recommends vitamin D (VD) supplementation to improve response to treatment of virtually all thyroid diseases, particularly AIHaT (5). Although it has been reported that reducing inflammatory damage and providing essential nutrients can improve thyroid function, sufficient clinical evidence does not support the routine prescription of nutritional supplements as part of HT treatment (6). In the present study, we investigated the clinical significance of some micronutrients known as essential for normal thyroid function—including selenium (Se), zinc (Zn), magnesium (Mg), vitamin A (VA), vitamin C (VC), and vitamin E (VE)—at physiological doses on thyroid function, metabolic parameters, and quality of life in patients receiving levothyroxine (LT4) and VD.

## Methods

### *Participant Eligibility Screening*

The current double-blind, randomized controlled trial was conducted among 104 outpatients with confirmed HT recruited from the endocrinology and metabolism institute of Iran University of Medical Sciences between January 2022 and May 2023. We selected individuals aged 18 to 69 years receiving scheduled LT4 doses as prescribed by an endocrinologist. We excluded individuals with a history of thyroid neoplasms, thyroidectomy or any serious/uncontrolled illness, current pregnant/breastfeeding women, current consumers of alcohol, and some drugs that interfere with thyroid hormones indices—including propranolol, corticosteroids, heroin, amphetamine, salicylates, phenylbutazone, aminoglutethimide, ethionamide, lithium, levodopa, cabergoline, bromocriptine, antioxidant or probiotic-containing supplements, any type of vitamin-mineral supplements except for regular doses of 50000 IU VD as prescribed by the endocrinologist.

### *Trial Design*

Eligible Patients signed the informed consent form. Endocrinologists had prescribed LT4 weekly for all patients. If the patients had not taken oral or injectable VD within the previous 3 to 6 months, they were given 8 pearls of 50000 IU VD (manufactured by Zahravi, Iranian Pharmaceutical Company) to use once per week; otherwise, they were given 2 pearls of 50000 IU VD to consume 1 per month during the study or advised to continue their VD injection plan. Participants were randomly assigned to either the LT4VDS (LT4+VD+supplements) group or the LT4VDP (LT4+VD+placebos) group. Those in the LT4VDS group were supplemented with 200 µg sodium selenite, 120 mg VC, 100 IU VE, 2500 IU VA + 1 tablet of 250 mg magnesium oxide per day + one capsule of zinc sulfate 25 mg 3 times a week, at least 2 hours after taking LT4 for 8 weeks. The LT4VDP group received placeboes similar in shape and color to the supplements above. All dietary supplements and placebos were produced by Iranian pharmaceutical firms (OPD Pharma, Jalinus, and Alhavi, respectively). Each participant's medication containers were coded with 1 to 3 random digits by a third party (specified by simple randomization) so that both the researcher and the participants were blinded to the codes of the 2 groups. A random chain of 90 repetitions of letters A and B (45 letters A and 45 letters B) was compiled by the statistical website sealedenvelope.com. This sequence was written on sheets of paper, respectively, and the sheets were placed in similar envelopes. The corresponding sequence number was remembered on the back of each envelope so that the envelope with the corresponding number was opened according to the entry order. During the intervention period, the participants were instructed to maintain their regular diet and level of physical activity. Compliance was evaluated via phone calls, and the number of remaining tablets or capsules in medication containers returned in follow-up visits. The phone number of the research team was given to the participants to report their questions or possible discomfort immediately. We excluded participants who discontinued LT4 or consumed <80% of supplements during the study period.

### *Physical Assessment and Interview*

Participants' sex, age, weekly LT4 dosage and other drugs used were recorded. At the beginning and end of the intervention, we assessed the type and quantity of their typically consumed foods and beverages using 2-day dietary recalls of 1 weekday and 1 weekend day. We subsequently calculated their dietary intake of energy and macro/micronutrient values using Nutritionist IV software. All participants' physical activity level (MET-min/week) was also measured using the International Physical Activity Questionnaire (IPAQ), short form. After the patients had rested for at least 5 minutes in the seated position, 2 readings for systolic and diastolic blood pressure (SBP and DBP) measurements were taken at 10-30-minute intervals using an automated device (Riester ri-Champion N, Germany). The 2 values were averaged in each of the 2 visits. In addition, we



[ DOI: 10.47176/mjiri.38.133 ]

measured the anthropometric values of each participant, including weight (wearing minimal clothing and no shoes), height, and waist and hip circumferences using a Japanese-made balance scale, a wall-mounted stadiometer, and a flexible measuring tape respectively. The most frequently used generic questionnaire in HT patients (7), a 36-item short-form survey, was used to evaluate the participants' quality of life. The survey included 8 subscales, as well as 2 general scores of physical and mental well-being. Using the scoring algorithm of the SF36 and z-score formulas, we computed and standardized each of the 8 scale scores. The subscales were multiplied by their respective score coefficients from the general US population to aggregate general scores, and then the 8 resulting products were added. Both aggregated components were ultimately transformed into norm-based scoring (8).

### *Laboratory Assessments*

We collected 21 cc of venous blood from each participant between 7:30 AM and 11:30 AM, before and after intervention, following a minimum 10-hour fast. Serum samples were separated by centrifugation at 3000×rpm for 15 minutes within 1 to 1.5 hours, whereas plasma samples (3 cc collected in an EDTA tube) were separated immediately by centrifugation at 3000×rpm for 10 min. All serum or plasma microtubes were stored at –80 °C until the analysis date. The enzyme-linked immunosorbent assay (ELISA) method was used to measure TSH, fT4, TPO-Ab, and fasting insulin concentrations (Pishtaz Teb and Monobind kits). Biochemical assessments, such as plasma fasting blood sugar (FBS), serum triglyceride (TG), serum total cholesterol (TC), serum low-density lipoprotein (LDL), and serum high-density lipoprotein (HDL) levels, were analyzed using biochemical kits Pars Azmoon, and PTS. Insulin resistance was computed before and after intervention using the homeostatic model assessment for insulin resistance (HOMA-IR) formula: fasting insulin*FBS/405. The inflammatory index C-reactive protein (CRP) was determined using an automated biochemistry analyzer (Pars Azmoon kit).

### *Sample Size Calculation, and Statistical Analysis*

G-power software (Version 3.1.2) was used to calculate the sample size. Standard deviations for the intervention and placebo groups were reported to be 1.24 and 2.35, respectively, based on the TSH value as the primary outcome in a previous study (9). We set the observed mean difference of TSH between the 2 groups as the desired effect size at 0.69. We considered the probability of type 1 error to be 5% ($\alpha = 0.05$) and type Π error to be 10% ($\beta = 0.1$, power = 90%). Resulting in a sample size of 45 patients in each group. We utilized SPSS (Version 26) for the statistical analysis. The Shapiro-Wilks test was used to determine the normality of the data distribution. The qualitative variables were compared using the chi-square or Fisher's exact test. We used independent sample t tests to compare parametric variables between the 2 groups and the Mann-Whitney U test for nonparametric data. We also compared the parameters change within each group using paired sample t tests or Wilcoxon ranked sign tests. For adjusted between-groups comparison of outcomes, we analyzed the covariance (ANCOVA) model for some baseline values.

## Results

The study's flowchart is depicted in Figure 1. None of the participants left the study due to clinical complications. The final analysis has been performed in 2 population sets—including 86 participants (per protocol) and 95 participants (intention to treat [ITT] "multiple imputation" method). We did not analyze ITT in the population set of 104 participants because we did not have access to

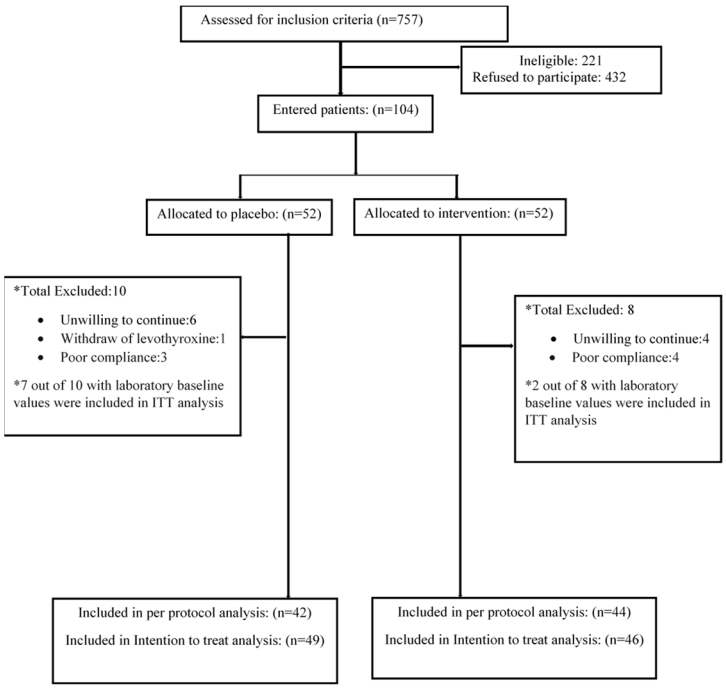


*Figure 1.* Flowchart of intervention





[ DOI: 10.47176/mjiri.38.133 ]

the baseline laboratory data of 9 participants (6 participants in the LT4VDS group, and 3 participants in the LT4VDP group).

As reported in Table 1, the baseline characteristics of participants were not significantly different between the 2 groups except for age. The participants who withdrew from the study did not differ from those who remained regarding age, body mass index (BMI), or weekly LT4 dose. According to baseline values, 22 of 42 participants in the LT4VDP group and 29 of 44 participants in the LT4VDS group were TPO-Ab ≥ 34 IU/mL, in which 13 and 17 individuals were TPO-Ab≥ 200 IU/mL, respectively. In both groups, 13 participants had TSH baseline values>4.2 mIU/L. The dietary values and physical activity did not differ significantly between groups at baseline and remained unchanged after the eighth week (Table 2). The results regarding thyroid function, quality of life, and metabolic indices are presented in Tables 3 and 4. In the LT4VDP group, higher postintervention values of FBS (effect size, 0.083) and HOMA-IR (effect size, 0.053) were observed. Intention to treat analysis showed similar results regarding FBS. In the tables, the asterisk denotes statistically significant within-group differences. The results demonstrated that several indices tended to increase within both groups during the intervention period, including fT4 ($P$ = 0.006 in the LT4VDP group and 0.013 in the LT4VDS group), TC ($P$ = 0.021 in the LT4VDP group, and $P$ = 0.016 in LT4VDS group), insulin ($P$ < 0.001 in LT4VDP group and $P$ = 0.041 in LT4VDS group), and mental quality of life ($P$ = 0.003 in LT4VDP group and $P$ = 0.002 in LT4VDS group). Also, increased HDL ($P$ = 0.008), FBS ($P$ = 0.014), HOMA-IR ($P$ < 0.001), and BMI ($P$ = 0.018) in the LT4VDP group as well as elevated physical quality of life in the LT4VDS group. In the LT4VDP group, a marginal increase regarding CRP, and insulin was observed, whereas in the LT4VDS group the physical quality of life partially improved (effect size, 0.012). Between-group comparison of difference values did not show significant results regarding other outcomes—including TSH, fT4, TPO-Ab, mental quality of life, TG, TC, LDL, HDL, SBP, and DBP (Figure 2).

*Table 1.* Baseline characteristics of participants

| Variable | | LT4VDP group (n=42) | LT4VDS group(n=44) | $P$ value |
|---|---|---|---|---|
| Age (year) | | 47.5 [10.8] | 42.1 [12] | 0.039[1] |
| Sex | Male | 2 (4.8%) | 5 (11.4%) | 0.414[2] |
| | Female | 40 (95.2%) | 39 (88.6%) | |
| LT4 dose (µgr/week) | | 500 [350, 700] | 675 [362.5, 700] | 0.086[1] |
| Disease duration (year) | | 7.5 [3.3, 15.5] | 7 [1.6, 14.2] | 0.443[1] |
| Baseline TPO-Ab>35 IU/mL | | 22 (52.4%) | 29 (65.9%) | 0.211[2] |
| VD supplementation | Weekly | 14 (33.3%) | 19 (43.2%) | 0.721[2] |
| | Monthly | 26 (61.9%) | 24 (54.5%) | |
| | Injection | 2 (4.8%) | 1 (2.3%) | |
| Taking statins | Yes | 8 (19%) | 4 (9.1%) | 0.282[3] |
| | No | 34 (81%) | 40 (90.9%) | |
| Taking anti-hypertensive drugs | Yes | 7 (16.7%) | 6 (13.6%) | 0.734[3] |
| | No | 35 (83.3%) | 38 (86.4%) | |
| Taking oral anti-diabetic agents | Yes | 7 (16.7%) | 2 (4.6%) | 0.083[2] |
| | No | 35 (83.3%) | 42 (95.4%) | |
| Taking antidepressants | Yes | 7 (16.7%) | 2 (4.6%) | 0.082[2] |
| | No | 35 (83.3%) | 42 (95.4%) | |
| Taking progesterone | Yes | 4 (9.5%) | 3 (6.8%) | 0.741[2] |
| | No | 38 (90.5%) | 41 (93.2%) | |

[1]: P values for independent sample t tests or Mann-Whitney U tests, [2] Fishers exact test, 3 Pearson's chi-square, LT4VDS: Levothyroxine+ Vitamin D+ Supplements group, LT4VDP: Levothyroxine+ Vitamin D+ Placebos group, LT4: Levothyroxine, TPO-Ab: anti-Thyroid Peroxidase Antibody, VD: vitamin D

*Table 2.* Comparison of physical activity level and dietary intake values between groups

| variable | | Baseline | Difference | $P$ v[1] | $P$ v[2] |
|---|---|---|---|---|---|
| PAL | LT4VDP | 635.6 [189.1, 1641.6] | 0 [-544.07, 288.4] | 0.361 | 0.775 |
| (MET-min/week) | LT4VDS | 548.4 [223.6, 1951.9] | -126.7 [-484.4, 90.4] | | |
| Energy | LT4VDP | 1845.4 [283.5] | 15.26 [163.5] | 0.332 | 0.098 |
| (kcal/day) | LT4VDS | 1958.3 [289.1] | 48.3 [179.4] | | |
| Se intake | LT4VDP | 44.3 [35.5, 58.8] | 2.3 [-4, 18] | 0.212 | 0.116 |
| (µg/day) | LT4VDS | 45.1 [37.6, 59.3] | 0 [-9.5, 3.1] | | |
| Zn intake (mg/day) | LT4VDP | 7.7 [1.4] | 0.09 [1.8] | 0.340 | 0.147 |
| | LT4VDS | 7.8 [1.4] | -0.2 [1.6] | | |
| Mg intake (mg/day) | LT4VDP | 230.7 [35.4] | 6.4 [37.2] | 0.211 | 0.617 |
| | LT4VDS | 222 [31.7] | 7.6 [38.4] | | |
| VA intake (µg/day) | LT4VDP | 697.4 [513.9, 1396.7] | 2.9 [-189.6, 275.9] | 0.773 | 0.163 |
| | LT4VDS | 814.9 [592.5, 1116.1] | 0.8 [-80.6, 248] | | |
| VC intake (mg/day) | LT4VDP | 82.2 [66.3, 96.8] | -2.4 [-22.1, 2.1] | 0.444 | 0.984 |
| | LT4VDS | 84.4 [74.8, 103.1] | -1.8 [-21.4, 1.3] | | |
| VE intake (mg/day) | LT4VDP | 15.9 [13.1, 19.2] | 0.01 [-0.8, 0.5] | 0.818 | 0.425 |
| | LT4VDS | 16.2 [15.1, 19.1] | -0.05 [-0.4, 2] | | |
| Iron intake | LT4VDP | 12.1 [2.1] | -0.03 [1.2] | 0.099 | 0.317 |
| (mg/day) | LT4VDS | 11.9 [2.2] | 0.01 [1.1] | | |

1: P values for between group comparison of difference values, using independent sample t tests or Mann-Whitney U tests, [2] ANCOVA tests with robust standard errors adjusted for baseline values, age and weekly levothyroxine dose as covariates, LT4VDS: Levothyroxine+ Vitamin D+ Supplements group, LT4VDP: Levothyroxine+ Vitamin D+ Placebos group, PAL: Physical Activity Level, MET: metabolic equivalent, Se: selenium, Zn: Zinc, Mg: Magnesium, VA: Vitamin A, VC: Vitamin C, VE: Vitamin E



[ DOI: 10.47176/mjiri.38.133 ]

*Table 3.* Comparison of thyroid function, Quality of life, and anthropometric values between groups

| Variable | | Baseline | After 8weeks | Difference | *P* v[1] | *P* v[2] | *P* v[3] |
|---|---|---|---|---|---|---|---|
| TSH | LT4VDP | 2.5 [1.1, 5.3] | 2.1 [0.7, 4.4] | 0 [-1.5, 0.6] | 0.072 | 0.312 | 0.571 |
| (mIU/L) | LT4VDS | 3.1 [0.5, 4.7] | 3 [1.4, 5.1] | 0 [-0.3, 1.2] | | | |
| fT4 | LT4VDP | 0.8 [0.7, 1.1] | 1 [0.8, 1.3] | 0.1* [0, 0.4] | 0.612 | 0.630 | 0.487 |
| (ng/dL) | LT4VDS | 0.9 [0.7, 1.2] | 1 [0.8, 1.3] | 0.1* [0, 0.3] | | | |
| TPO-Ab | LT4VDP | 43.3 [1.8, 286.2] | 25.1 [3.7, 281.2] | 1.4 [-4.5, 21.7] | 0.932 | 0.914 | 0.771 |
| (IU/mL) | LT4VDS | 102.5 [10.1, 397.7] | 76.5 [8.4, 330] | 2.1 [-16.5, 37.5] | | | |
| Ph-QoL | LT4VDP | 41.8 [9.7] | 42.1 [10.2] | 0.2 [9.1] | 0.044 | 0.021 | 0.314 |
| | LT4VDS | 43.2 [8.5] | 46.9 [7.8] | 3.6 [6.3]* | | | |
| M-QoL | LT4VDP | 41.3 [31.7, 50] | 48.7 [38.3, 54.7] | 5.8 [11.9]* | 0.672 | 0.310 | 0.394 |
| | LT4VDS | 40 [30, 45.3] | 45.5 [35.5, 52.1] | 4.6 [8.9]* | | | |
| BMI | LT4VDP | 28.9 [24.8, 31.5] | 28.9 [24.9, 31.4] | 0.1* [0, 0.5] | 0.741 | 0.944 | 0.401 |
| ($kg/m^2$) | LT4VDS | 27.6 [23.6, 31.1] | 28.2 [23.9, 31.5] | 0.1 [-0.2, 0.5] | | | |
| WC (cm) | LT4VDP | 93.7 [85, 103.2] | 96 [87.6, 104.2] | 0.5[-1.5, 2] | 0.537 | 0.556 | 0.724 |
| | LT4VDS | 94.5 [84, 100.7] | 92.7 [86, 101.7] | 0 [-3, 3] | | | |

1: P values for between group comparison of difference values, using independent sample t tests or Mann-Whitney U tests, 2 ANCOVA tests with robust standard errors adjusted for baseline values, age, and weekly levothyroxine dose
as covariates, 3 Intention to treat analysis, "Multiple imputation" method adjusted for baseline values, age, weekly levothyroxine dose, physical activity level, energy intake, and vitamin D supplementation as covariates *$P$value< 0.05 for within group test, using paired sample t tests or Wilcoxon ranked sign tests. LT4VDS: Levothyroxine+ Vitamin D+ Supplements group, LT4VDP: Levothyroxine+ Vitamin D+ Placebos group, TSH: Thyroid Stimulating Hormone, fT4: free Thyroxine, TPO-Ab: anti-Thyroid Peroxidase Antibody, Ph-QoL: Physical Quality of Life, M-QoL: Mental Quality of Life, BMI: Body Mass Index, WC: Waist Circumference.

*Table 4.* Comparison of lipid and glycemic indices, blood pressure, and inflammatory state between LT4VDS and LT4VDP groups

| Variable | | Baseline | After 8 weeks | Difference | *P* v[1] | *P* v[2] | *P* v[3] |
|---|---|---|---|---|---|---|---|
| TG (mg/dL) | LT4VDP | 105.5 [81, 149.2] | 105 [80.7, 144.2] | -2.5 [-27.2, 23.5] | 0.351 | 0.710 | 0.441 |
| | LT4VDS | 117 [74, 145] | 102 [82.7, 160] | 11.5 [-20.5, 30.5] | | | |
| TC (mg/dL) | LT4VDP | 171.5 [146.7, 194.7] | 170.5 [157.7, 205.2] | 12.5* [-6.5,28.2] | 0.712 | 0.445 | 0.667 |
| | LT4VDS | 168 [150, 205] | 184 [164, 208.7] | 18*[-2.5, 27.7] | | | |
| LDL | LT4VDP | 97.5 [83, 129] | 105.5 [81.2, 123.2] | 6.5 [-8, 14] | 0.920 | 0.421 | 0.502 |
| (mg/dL) | LT4VDS | 102 [82.5, 127.2] | 108.5 [90, 120] | 7 [-6.2, 14.7] | | | |
| HDL | LT4VDP | 52.1 [10.2] | 56.4 [10.9] | 2.5*[-1.2, 11] | 0.114 | 0.090 | 0.258 |
| (mg/dL) | LT4VDS | 50.3 [10.7] | 51.7 [10.5] | 0 [-6.5, 7.5] | | | |
| FBS | LT4VDP | 88.5 [83, 100] | 98.5 [85.7, 106.5] | 9.5* [-6.5, 16.2] | 0.131 | 0.012 | 0.011 |
| (mg/dL) | LT4VDS | 85 [80, 93] | 89 [82.5, 95.7] | 2.5 [-5.5, 8.2] | | | |
| Insulin | LT4VDP | 6.6 [4.9, 9.5] | 8.6 [6.3, 15.2] | 2.9* [0.4, 6.1] | 0.042 | 0.111 | 0.443 |
| (µIU/mL) | LT4VDS | 6.9 [4.3, 10.4] | 7.6 [5.1, 11.3] | 1* [-0.5, 2.3] | | | |
| HOMA-IR | LT4VDP | 1.4 [0.9, 2.2] | 2.1 [1.3, 3.8] | 0.7* [0, 1.6] | 0.011 | 0.031 | 0.080 |
| | LT4VDS | 1.4 [0.8, 2.3] | 1.6 [1.1, 2.4] | 0.1 [-0.2, 0.5] | | | |
| CRP | LT4VDP | 2 [2, 3] | 3 [2, 4] | 1 [-1, 1] | 0.051 | 0.220 | 0.091 |
| (mg/dL) | LT4VDS | 2 [1.2, 3] | 2 [1, 3] | -0.6 [-1, 1] | | | |
| SBP | LT4VDP | 11.3 [10.4, 13.2] | 11.1 [10.2, 12.6] | -0.1[1] | 0.623 | 0.737 | 0.549 |
| (mmHg) | LT4VDS | 11.2 [10.3, 12.1] | 11.1 [10.6, 11.7] | 0 [0.9] | | | |
| DBP | LT4VDP | 7.7 [1] | 7.7 [1] | 0 [0.5] | 0.524 | 0.710 | 0.832 |
| (mmHg) | LT4VDS | 7.4 [0.9] | 7.3 [0.8] | -0.1[0.7] | | | |

[1]: P values for between group comparison of difference values, using independent sample t tests or Mann-Whitney U tests, [2] ANCOVA tests with robust standard errors adjusted for baseline values, age, and weekly levothyroxine dose as covariates, 3 Intention to treat analysis, "Multiple imputation" method adjusted for baseline values, age, weekly levothyroxine dose, physical activity level, energy intake, and vitamin D supplementation as covariates. *$P$ value< 0.05 for within group test, using paired sample t tests or Wilcoxon ranked sign tests. LT4VDS: Levothyroxine+ Vitamin D+ Supplements group, LT4VDP: Levothyroxine+ Vitamin D+ Placebos group, TG: Triglyceride, TC: Total Cholesterol, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, FBS: Fasting Blood Sugar, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance ((FBS*Insulin)/405), CRP: C-Reactive Protein , SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure

## Discussion

The present study showed that in HT patients, supplementation of Se, Zn, Mg, VA, VC, and VE at physiological doses for 8 weeks had no greater effect on major thyroid function indices (TSH, fT4, TPO-Ab), lipid indices (TG, TC, LDL, HDL), blood pressure indices (SBP, DBP), anthropometric indices (BMI, and WC), CRP, and mental quality of life compared with treatment with LT4 and VD. Nevertheless, controlling effects on FBS, HOMA-IR, and partially improved physical quality of life were observed versus placebo. Moreover, both of our VD-supplemented groups demonstrated an equal increase in fT4 compared with the baseline.

Recent evidence reported that Se intake ≥ 200 µg per day for ≥3 months demonstrated greater efficacy in reducing TPO-Ab than LT4 monotherapy or placebo in AIHaT patients, despite having negligible clinical effects on TSH (10, 11). Two randomized clinical trials were conducted in the same geographical region as ours (ie, the same Se content of the country's soil and its bioavailability to enter the food chain), administering 200 µg Se for 3 months to AIHaT patients with (12) or without (13) LT4 therapy were in agreement with the summary of other clinical results. Our results are unfavorable regarding TPO-Ab, most likely because a longer intervention duration of Se therapy seems to be beneficial in TPO-Ab significant decrement. Our findings were comparable to those of Krysiak et al who found no beneficial effect of 200 µg/day selenomethionine supplementation added to LT4 versus LT4 alone for 3 months on TPO-Ab, TSH, and fT4 in AIHaT patients (14). Se supplementation (200 µg, for 3-6 months) is also reported to improve the quality of life in AIHaT patients with HT (15); consequently, Se may influence the elevated quality of life indices in the LT4VDS group. Our finding regarding the





[ DOI: 10.47176/mjiri.38.133 ]

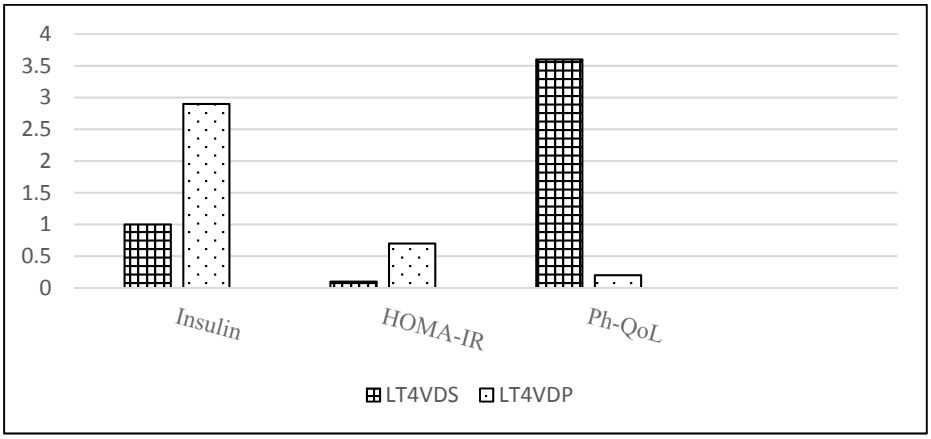


*Figure 2.* *P* value≤ 0.05 for between group comparison of difference values, using independent sample t tests or Mann-Whitney U tests

LT4VDS: Levothyroxine+ Vitamin D+ Supplements group, LT4VDP: Levothyroxine+ Vitamin D+ Placebos group, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance ((FBS*Insulin)/405), CRP: C-Reactive Protein, Ph-QoL: Physical Quality of Life

mental quality of life is consistent with a pilot study among euthyroid women with AIHaT; consuming daily 4000 IU VD or 200 µg selenomethionine for 6 months resulted in mood improvement in both groups compared with untreated AIHaT patients, with VD having a greater effect than Se (16). We found an increase in mental quality of life compared with baseline, but there was no statistically significant difference between the 2 groups (consuming VD) after the intervention.

In the same geographical region as ours, 68 overweight or obese HT women were divided into 4 groups, including 30 mg Zn/day + 200 µg Se/day, 30 mg Zn/day + placebo, 200 µg Se/day + placebo, and placebo + placebo for 12 weeks. Ultimately, there were no significant differences in BMI, serum TSH, fT4, and Zn and Se levels. Still, the first group showed significant improvement in TSH and fT4 compared with corresponding baseline values (9). The preintervention sufficient values of serum Zn and Se are a vital point to note in the previously cited article. Although we did not assess Se and Zn levels biochemically, our participants' dietary intake amounts were close to the recommended values. We administered a Zn supplement dose half that of Mahmoodianfard et al (9) in a shorter period; however, our overall results were comparable between groups regarding thyroid and anthropometry values.

Magnesium appears to affect the improved physical quality of life in the LT4VDS group, consistent with the hypothesis that magnesium affects psychosomatic or medically unexplained symptoms in HT patients (17-19). Rabbani et al reported significant improvement in fT4, high sensitive-CRP, and BMI, but no improvement in TSH among HT patients consuming 30 mg Zn/day + 250 mg Mg/day, + 25,000 IU VA twice/week for 10 weeks versus the placebo group (20). They attributed BMI reduction in their clinical trial to the regulatory role of VA in adipose tissue. We found no difference in postintervention fT4 results between the 2 groups, however, we were not able to compare these effects versus placebo, due to the VD consumption in both groups. Contrary to the LT4VDS group, the BMI increased significantly compared to the baseline in the LT4VDP group. Our results in the inflammatory state may be attributable to the less sensitive index we employed; additionally, we administered approximately half the dose of Zn and 35% of the dose of VA (close to the DRI for adults) per day and for a shorter duration than Rabbani et al. We also utilized a quarter dose of VC for a shorter duration than the study by Karimi et al (13), which compared the effects of a 3-month daily intake of 500 mg VC versus 200 µg Se in AIHaT patients without LT4 therapy. TPO-Ab and TSH levels did not differ between groups; however, TPO-Ab levels decreased in both groups from baseline.

A slight increase in CRP and BMI, as conditions associated with HT, among the LT4VDP group may have stimulated glycemic values elevation within the normal range. According to the summary of clinical findings, Zn, Mg, VE, and Se likely played preventative roles against the deterioration of postintervention glycemic results in the LT4VDS group. furthermore, Zn, VC, and VE likely prevented the CRP elevation during 8 weeks of intervention among the LT4VDS group.

Due to increasing attention on the beneficial roles of VD in HT, fortunately, this vitamin is routinely taken in many HT patients. Therefore, we decided not to limit the sampling of the present study to the condition of VD deficiency in participants. Two randomized clinical trials were conducted in the same geographical region as ours (ie, the same latitude, which affects VD production through the skin) and administering 50000 IU VD once per week for 3 months to AIHaT patients with LT4 therapy and serum VD insufficient status found no significant reduction in TSH (21, 22), TPO-Ab, and T4 (23), most likely because, regardless of the VD dose administered and the basic level of serum 25(OH) D, TPO-Ab levels have been reported to significantly decrease when the supplementation continues for over 3 months (24, 25).

Due to financial constraints, we could not evaluate all study participants for the serum 25(OH) VD. Per the World Health Organization (WHO) recommendations for VD consumption, we advised all participants to maintain serum 25(OH)D levels within the normal range. If we had been able to extend the duration of our study, we would have found some beneficial effects of Se + VD supplementation



[ DOI: 10.47176/mjiri.38.133 ]

on TPO-Ab and more ameliorated metabolic factors in response to Zn and Mg supplementation. In addition, depending on the type of supplement available, we used sodium selenite, an inorganic form of Se that is less bioavailable than seleno-cysteine and seleno-methionine. We used a nonspecific marker to evaluate inflammatory conditions, due to availability and cost limitations, which may not have been sensitive enough.

We suggest that future nutritional intervention studies be conducted in patients with subclinical HT (increased TSH with normal fT4), new cases of HT (beginning treatment with LT4), and those who have received a fixed dose of LT4 for at least 3 months. According to the evidence summary presented in this study, interventions should last longer than 3 months. Furthermore, it is hypothesized that longer durations of VD therapy may be advantageous for normalizing TPO-Ab in regions comparable to ours.

## Conclusion

Cosupplementation with Se, Zn, Mg, VA, VC, and VE may affect certain clinical outcomes in HT patients concurrently receiving regular doses of LT4 and VD. Future clinical trials should be designed to last longer than our trial to achieve the desired outcomes.

### *Authors' Contributions*

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Mehrnaz Nikkhah, Parvin Sarbakhsh, and Fariba Alaei-Shahmiri. The first draft of the manuscript was written by Mehrnaz Nikkhah and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### *Ethical Considerations*

Approval was granted by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1400.986). The Iranian Registry of Clinical Trials number: IRCT20200610047719N1.

### *Acknowledgment*

The authors express their gratitude to the participants who participated in this study.

### *Conflict of Interests*

The authors declare that they have no competing interests.

## References

1. Wang Y, Sun Y, Yang B, Wang Q, Kuang H. The management and metabolic characterization: hyperthyroidism and hypothyroidism. Neuropeptides. 2022:102308.
2. Tellechea ML. Meta-analytic evidence for increased low-grade systemic inflammation and oxidative stress in hypothyroid patients. Can levothyroxine replacement therapy mitigate the burden? Endocrine. 2021;72(1):62-71.
3. Mincer DL, Jialal I. Hashimoto Thyroiditis. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Ishwarlal Jialal declares no relevant financial relationships with ineligible companies.: StatPearls Publishing Copyright © 2023, StatPearls Publishing LLC.; 2023.
4. Perros P, Van Der Feltz-Cornelis C, Papini E, Nagy EV, Weetman AP, Hegedüs L. The enigma of persistent symptoms in hypothyroid patients treated with levothyroxine: A narrative review. J Clin Endocrinol. 2023;98(4):461-8.
5. Babić Leko M, Jureško I, Rozić I, Pleić N, Gunjača I, Zemunik T. Vitamin D and the Thyroid: A Critical Review of the Current Evidence. Int J Mol Sci. 2023;24(4):3586.
6. Winther KH, Papini E, Attanasio R, Negro R, Hegedüs L. A 2018 European Thyroid Association Survey on the Use of Selenium Supplementation in Hashimoto's Thyroiditis. Eur Thyroid J. 2020;9(2):99-105.
7. Borson-Chazot F, Terra J-L, Goichot B, Caron P. What is the quality of life in patients treated with levothyroxine for hypothyroidism and how are we measuring it? A critical, Narrative review. J Clin Med. 2021;10(7):1386.
8. Ware J, Kosinski M, Keller S. SF-36 physical and mental health summary scales. A user's manual. 2001;1994.
9. Mahmoodianfard S, Vafa M, Golgiri F, Khoshniat M, Gohari M, Solati Z, et al. Effects of zinc and selenium supplementation on thyroid function in overweight and obese hypothyroid female patients: a randomized double-blind controlled trial. J Am Coll Nutr. 2015;34(5):391-9.
10. Qiu Y, Xing Z, Xiang Q, Yang Q, Zhu J, Su A. Insufficient evidence to support the clinical efficacy of selenium supplementation for patients with chronic autoimmune thyroiditis. Endocrine. 2021;73:384-97.
11. Wang YS, Liang SS, Ren JJ, Wang ZY, Deng XX, Liu WD, et al. The Effects of Selenium Supplementation in the Treatment of Autoimmune Thyroiditis: An Overview of Systematic Reviews. Nutrients. 2023;15(14).
12. Kachouei A, Rezvanian H, Amini M, Aminorroaya A, Moradi E. The effect of levothyroxine and selenium versus levothyroxine alone on reducing the level of anti-thyroid peroxidase antibody in autoimmune hypothyroid patients. Adv Biomed Res. 2018;7.
13. Karimi F, Omrani G. Effects of selenium and vitamin C on the serum level of antithyroid peroxidase antibody in patients with autoimmune thyroiditis. J Endocrinol Invest. 2019;42:481-7.
14. Krysiak R, Okopien B. The effect of levothyroxine and selenomethionine on lymphocyte and monocyte cytokine release in women with Hashimoto's thyroiditis. J Clin Endocrinol Metab. 2011;96(7):2206-15.
15. Filipowicz D, Majewska K, Kalantarova A, Szczepanek-Parulska E, Ruchała M. The rationale for selenium supplementation in patients with autoimmune thyroiditis, according to the current state of knowledge. Endokrynol Polska. 2021;72(2):153-62.
16. Krysiak R, Kowalcze K, Szkróbka W, Okopień B. Sexual Function and Depressive Symptoms in Young Women with Euthyroid Hashimoto's Thyroiditis Receiving Vitamin D, Selenomethionine and Myo-Inositol: A Pilot Study. Nutrients. 2023;15(12):2815.
17. Moncayo R, Moncayo H. Exploring the aspect of psychosomatics in hypothyroidism: the WOMED model of body–mind interactions based on musculoskeletal changes, psychological stressors, and low levels of magnesium. Woman-Psychosomatic Gynaecology and Obstetrics. 2014;1:1-11.
18. Moncayo R, Moncayo H. The WOMED model of benign thyroid disease: acquired magnesium deficiency due to physical and psychological stressors relates to dysfunction of oxidative phosphorylation. BBA Clin. 2015;3:44-64.
19. Moncayo R, Moncayo H. Proof of concept of the WOMED model of benign thyroid disease: Restitution of thyroid morphology after correction of physical and psychological stressors and magnesium supplementation. BBA Clin. 2015;3:113-22.
20. Rabbani E, Golgiri F, Janani L, Moradi N, Fallah S, Abiri B, et al. Randomized study of the effects of zinc, vitamin A, and magnesium co-supplementation on thyroid function, oxidative stress, and hs-CRP in patients with hypothyroidism. Biol Trace Elem Res. 2021;199:4074-83.
21. Chahardoli R, Saboor-Yaraghi A-A, Amouzegar A, Khalili D, Vakili AZ, Azizi F. Can supplementation with vitamin D modify thyroid autoantibodies (Anti-TPO Ab, Anti-Tg Ab) and thyroid profile (T3, T4, TSH) in Hashimoto's thyroiditis? A double blind, Randomized clinical trial. Hormone Metab Res. 2019;51(05):296-301.
22. Anaraki PV, Aminorroaya A, Amini M, Momeni F, Feizi A, Iraj B, et al. Effect of Vitamin D deficiency treatment on thyroid function and autoimmunity markers in Hashimoto's thyroiditis: A double-blind randomized placebo-controlled clinical trial. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2017;22.
23. Chaudhary S, Dutta D, Kumar M, Saha S, Mondal SA, Kumar A, et





[ DOI: 10.47176/mjiri.38.133 ]

al. Vitamin D supplementation reduces thyroid peroxidase antibody levels in patients with autoimmune thyroid disease: An open-labeled randomized controlled trial. Indian J Endocrinol Metab. 2016;20(3):391.
24. Zhang J, Chen Y, Li H, Li H. Effects of vitamin D on thyroid autoimmunity markers in Hashimoto's thyroiditis: systematic review and meta-analysis. J Int Med Res. 2021;49(12):03000605211060675.
25. Wang S, Wu Y, Zuo Z, Zhao Y, Wang K. The effect of vitamin D supplementation on thyroid autoantibody levels in the treatment of autoimmune thyroiditis: a systematic review and a meta-analysis. Endocrine. 2018;59:499-505.



[ DOI: 10.47176/mjiri.38.133 ]